

Reexamination of Cyclodextrin-Induced Conformational Enantiomerism of Bilirubin in Aqueous Solution

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A cyclodextrin (CDx)-induced conformational enantiomerism of bilirubin IXα in dianionic form (BR^{2-}) was reinvestigated to establish the mechanism for complexation of BR^{2-} with native CDxs, such as α-, β-, and γ-CDxs, in aqueous solution. Since previous studies have lacked NMR examinations, we concentrated our effort on measuring the ^{1}H NMR spectra to determine the structure of the BR^{2-}–CDx complex, where BR^{2-} takes the (*M*)-helix conformation. Circular dichroism (CD) spectroscopy was also utilized. ^{1}H NMR measurements including ROESY suggest the shallow penetration of a hydrophobic part of BR^{2-} into the CDx cavity. ^{1}H NMR data also show adsorption of the BR^{2-} dianion on the outside wall of the CDx cavity. Although BR^{2-} is adsorbed on the cavities of heptakis(2,3-di-*O*-methyl)- and heptakis(2,6-di-*O*-methyl)-β-CDxs, no enantiomerism occurred when these *O*-methylated β-CDxs were used in place of native CDxs. In contrast, heptakis(6-*O*-methyl)-β-CDx exhibits a definite (−)-to-(+) bisignate CD spectrum due to preferencial formation of (*M*)-helix BR^{2-}. These results are interpreted in terms that the BR^{2-} dianion is adsorbed on the outside wall of the asymmetrically twisted CDx cavity, and is anchored through two-point hydrogen bonding between the CO_2^- group(s) of BR^{2-} and the OH groups at the 2- and 3-positions of the CDx.

(4*Z*, 15*Z*)-Bilirubin-IXα (BR) is a bile pigment produced by the metabolism of heme, where two dipyrrinone chromophores are linked to each other by a methylene bridge.[1–3] Intramolecular hydrogen bonding causes the formation of the (*P*)- and (*M*)-helix conformational enantiomers of BR in organic solvents (Fig. 1). It has been assumed that the dissociated BR (BR^{2-}) also forms intramolecular hydrogen bonds between the CO_2^- groups and the pyrrole NH parts of BR^{2-} in aqueous solution. Fast interconversion between the (*P*)- and (*M*)-enantiomers occurs in homogeneous solution. However, it is possible to enrich the distribution of one of the enantiomers by enantioselective complexation with a chiral host compound in aqueous solution. For example, BR^{2-} becomes optically active when it is bound to serum albumin,[4–8] deoxycholate micelles,[9,10] chiral cyclophanes,[11] nucleosides,[12] octyl β-D-maltoside micelles,[13] non-cyclic oligosaccharides,[14] and native cyclodextrins (CDx)[15,16] in aqueous solutions. Such conformational enantiomerism of BR^{2-} has mostly been investigated by means of circular dichroism (CD) spectroscopy. The exciton-coupling theory[17] predicts that BR^{2-} having (*P*)-helicity shows a (+)-to-(−) bisignate CD Cotton effect (plus and minus CD signals at longer and shorter wavelengths, respectively) and one with (*M*)-helicity exhibits a (−)-to-(+) bisignate signal. The circular dichroisms ($\Delta\varepsilon$) of BR^{2-} (3×10^{-5} mol dm^{-3}) in 0.1 mol dm^{-3} Tris buffer at pH 7.0 in the presence of 1×10^{-2} mol dm^{-3} β-CDx are −4.0 and +3.0 dm^3 mol^{-1} cm^{-1} at 498 and 427 nm, respectively.[15] Such a weak (−)-to-(+) bisignate CD signal suggests that a β-CDx host slightly enriches the (*M*)-enantiomer of BR upon complexation. Meanwhile, the (−)-to-(+) bisignate CD signal of BR in dichloromethane containing optically active quinine ([BR]/[quinine] = 1/300) is much stronger than that for the BR^{2-}–β-CDx system in aqueous solution, the $\Delta\varepsilon$ values being −131 and +74 dm^3 mol^{-1} cm^{-1} at 465 and 412 nm, respectively.[18] In contrast, the CD signals of the BR dimethyl ester in dichloromethane in the presence of quinine are quite weak, the $\Delta\varepsilon$ values being +1.7 and −3.3 dm^3 mol^{-1} cm^{-1} at 470 and 415 nm, respectively.[18] These results strongly suggest the participation of acid–base salt complex formation in the conformational enantiomerism of BR by quinine. In other words, two CO_2H groups of BR play an important role for quinine-induced conformational enantiomerism. In spite of numerous reports on the conformational enantiomerism of BR and BR^{2-}, few studies on the mechanism for this phenomenon have been presented. Lightner and his co-workers assumed that the intramolecularly hydrogen-bonded BR^{2-} (CO_2^-···

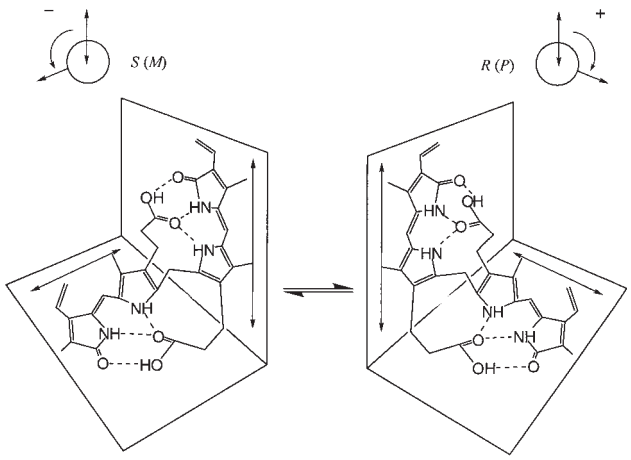

Fig. 1. Conformational enantiomers of BR in the acidic form.

HN) enantioselectively complexes with native CDxs.[15] Resonance Raman spectroscopy supports such a complex.[19] On the other hand, we previously reported that the bisignate CD signal of BR^{2-} is enhanced when cyclooctanol is included into the β-CDx cavity and that no CD signal is observed when heptakis(2,3,6-tri-*O*-methyl)-β-CDx (TMe-β-CDx) is used in place of native β-CDx.[16] In addition, the fact that the effect of heptakis(6-deoxy)-β-CDx is the same as that of β-CDx suggests the essential role of the secondary OH groups of β-CDx on the conformational enantiomerism of BR^{2-}. Based on these findings, we claimed that the intermolecular hydrogen bonds between the secondary OH groups of CDx and the CO_2^- groups of BR^{2-} participate in conformational enantiomerism, though no direct evidence was obtained.[16] Similar hydrogen-bonded complexes have been assumed for the BR^{2-}-steroid system.[20] However, it has generally been known that a hydrogen bond formed in an organic solvent is easily dissociated upon the addition of protic polar solvents, such as water and methanol. We previously studied a hydrogen-bonding interaction of the *p*-methylbenzoate anion with native CDxs in DMSO-d_6.[21] Relatively stable hydrogen-bonded complexes of the carboxylate anion and native α-, β-, and γ-CDxs are formed in DMSO-d_6. However, the addition of 1–2%(v/v) D_2O causes a dissociation of the hydrogen-bonded complexes. Do such results indicate the absence of intermolecular hydrogen bonding in complexation of BR^{2-} with native CDxs in aqueous solution? The answer might be "no". A typical example of hydrogen bonding between the CO_2^- and OH groups in aqueous solution is the *o*-hydroxybenzoate anion. The lower pK_1 and higher pK_2 values of *o*-hydroxybonzoic acid clearly indicate the intramolecular hydrogen bond, as shown in Fig. 2. Therefore, it might be possible to form a hydrogen bond if a hydrogen-bond acceptor is located in the vicinity of hydrogen-bond donor (proximity effect). The inclusion of BR^{2-} into the CDx cavity may provide such a proximity effect. The present study deals with the participation of hydrogen bonding in the CDx-induced conformational enantiomerism of BR^{2-} in aqueous solution.

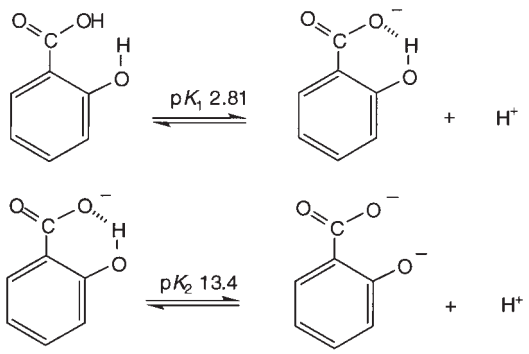

Fig. 2. Intramolecular hydrogen bond of *o*-hydroxybenzoate anion and pK_a values of *o*-hydroxybenzoic acid.

Results

CD Spectra—Effects of Alkanols. Table 1 gives the intensities of the (−)-to-(+) bisignate CD signal of BR^{2-} in water at pH 10.8 (NaOH) in the presence of α-, β- and γ-CDxs and various alkanols. At pH 10.8, two carboxy groups of BR^{2-} are dissociated. As reported by Lightner et al., native CDxs induce the conformational enantiomerism of BR^{2-}, where (*M*)-BR^{2-} is preferetially formed.[15] In the absence of alkanol, the CD intensity of BR^{2-} increases in the order α- < β- < γ-CDxs.

In the cases of α- and β-CDxs, the addition of 1-alkanols, such as 1-butanol, 1-pentanol, and 1-hexanol, causes an increase in the CD intensity of BR^{2-}. These 1-alkanols are known to be included into the cavities of α- and β-CDxs.[22] Meanwhile, no effect of these 1-alkanols was observed with γ-CDx, whose cavity size is too large to include these guests tightly. In contrast, the CD intensity of BR^{2-} in the presence of γ-CDx increases upon the addition of more bulky cycloalkanols, such as cyclohexanol, cycloheptanol, and cyclooctanol. The same effects of cycloalkanols were observed with β-CDx. The present study clearly exhibits the relationship between the cavity size of CDx and the size of alkanol on conformational enantiomerism of BR^{2-}. The CDx including an alkanol tightly induces the conformational enantiomerism of BR^{2-} effectively.

^{1}H NMR Spectra—Role of Inclusion for Conformational Enantiomerism. The effects of the alkanols clearly indicate that deep inclusion of the BR^{2-} molecule into the host cavity is not essential for conformational enantiomerism. Then, a ^{1}H NMR study was carried out to understand the role of the CDx cavity. No detailed NMR study has been reported with the CDx-induced enantiomerism of BR^{2-} so far. Figure 3 shows the ^{1}H NMR spectra of β-CDx in D_2O in both the absence and presence of BR^{2-} together with the complexation-induced shifts (CIS, $\Delta\delta$) in the chemical shifts of the protons of β-CDx. All of the proton signals of β-CDx slightly shift to

Table 1. Circular Dichroisms ($\Delta\varepsilon$) of BR^{2-} (2.5×10^{-5} mol dm^{-3}) in Aqueous Solution at pH 10.8 (NaOH) in the Presence of α-, β-, and γ-CDxs (1×10^{-2} mol dm^{-3}) and Effects of Added Alkanols (1×10^{-2} mol dm^{-3})

Alkanol	α-CDx		β-CDx		γ-CDx	
(*K* for α-CDx, *K* for β-CDx)[a]	$\Delta\varepsilon_1$/nm	$\Delta\varepsilon_2$/nm	$\Delta\varepsilon_1$/nm	$\Delta\varepsilon_2$/nm	$\Delta\varepsilon_1$/nm	$\Delta\varepsilon_2$/nm
none	−3.1 (460)	+3.4 (403)	−7.4 (454)	+4.9 (401)	−8.1 (457)	+5.9 (407)
1-butanol (89.1, 16.6)	−6.9 (455)	+5.1 (410)	−9.5 (455)	+6.8 (405)	−9.8 (454)	+6.2 (403)
1-pentanol (324, 63.1)	−11.7 (459)	+9.1 (404)	−12.9 (456)	+9.8 (406)	−9.4 (456)	+6.5 (402)
1-hexanol (891, 219)	−9.9 (459)	+9.3 (408)	−16.6 (457)	+13.7 (407)	−8.2 (454)	+7.2 (405)
cyclohexanol (64.6, 501)	−3.5 (458)	+3.6 (403)	−16.7 (456)	+11.5 (406)	−11.0 (456)	+7.7 (408)
cycloheptanol (79.4, 1700)	−4.8 (459)	+3.7 (402)	−25.0 (459)	+20.8 (408)	−17.0 (456)	+13.1 (403)
cyclooctanol (234, 2000)	−3.6 (459)	+3.6 (402)	−29.4 (459)	+23.2 (407)	−36.4 (454)	+27.5 (404)

a) The data of the binding constants (*K*, dm^3 mol^{-1}) for complexation of the alkanols with CDx are cited from Ref. 22.

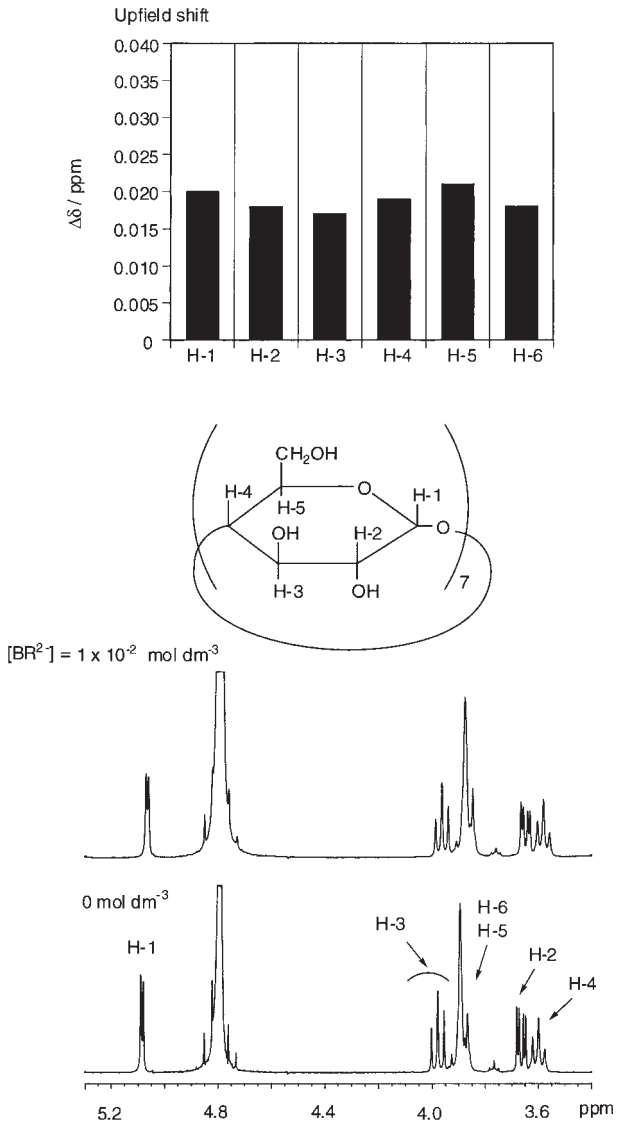

Fig. 3. ^{1}H NMR spectra of β-CDx (4×10^{-3} mol dm^{-3}) in D_2O in the absence and the presence of BR^{2-} (1×10^{-2} mol dm^{-3}) at pD 10.8 and 25 °C and CIS of β-CDx.

higher magnetic fields to a similar extent upon complexation. There have been many ^{1}H NMR studies on the inclusion of guests into CDx cavities. In many cases, the signals of the protons at the 1-, 2-, and 4-positions of CDxs are scarcely influenced by the included guests.[23] The CIS data showing up-field shifts of all protons of β-CDx suggest that the BR^{2-} molecule is adsorbed on the outside wall of the β-CDx cavity.

Figure 4 shows the ^{1}H NMR spectra of BR^{2-} (4×10^{-3} mol dm^{-3}) in D_2O at pD 10.8 in both the absence and presence of β-CDx (1.2×10^{-2} mol dm^{-3}). The assignment of the signals due to BR^{2-} was performed while referring to the previous papers.[24,25] No shifts in the chemical shifts of BR^{2-} were observed upon the addition of β-CDx. Since the binding constant (K) for the complexation of BR^{2-} with β-CDx was reported to be 23 dm^3 mol^{-1},[16] the concentration of the BR^{2-}–β-CDx complex was calculated to be 1×10^{-3} mol dm^{-3} under the present conditions. About a fourth of the added BR^{2-} dianion is transformed to the complex. The

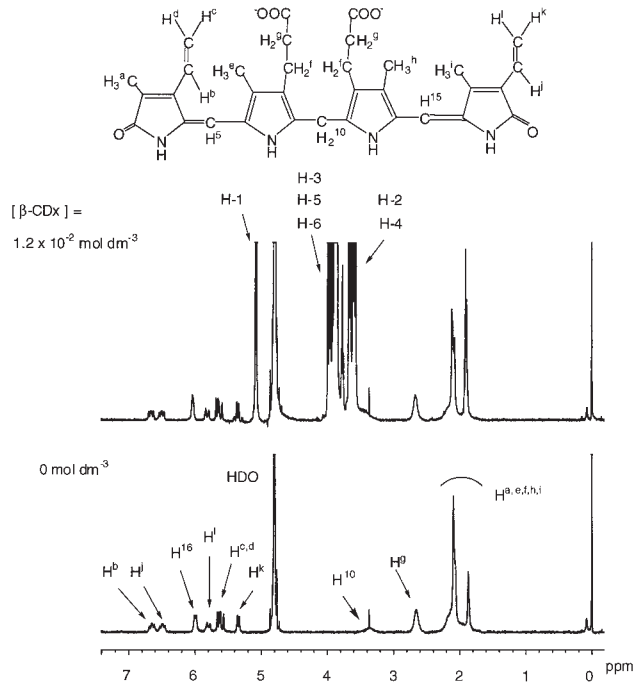

Fig. 4. ^{1}H NMR spectra of BR^{2-} (4×10^{-3} mol dm^{-3}) in D_2O in the absence and the presence of β-CDx (1.2×10^{-2} mol dm^{-3}) at pD 10.8 and 25 °C.

signal due to the proton located in the vicinity of the CO_2^- group tends to shift to a higher magnetic field upon complexation with CDx.[23] However, no shift was observed with the protons due to the propionate residues (H^g) of BR^{2-}.

The ROESY spectrum for the BR^{2-}–β-CDx system is shown in Fig. 5. The characteristic point in the ROESY spectrum is the correlation between the protons at the 3-, 5-, and 6-positions of β-CDx and those of the methyl protons as well as the protons of the vinyl groups of BR^{2-}. Such a correlation suggests that a hydrophobic part of BR^{2-} penetrates into the CDx cavity to some extent from both sides of the host cavity, though the ^{1}H NMR signals due to BR^{2-} are scarcely affected by β-CDx. The ROESY spectrum indicates, at least, that two types of the BR^{2-}–β-CDx complexes are formed in this system.

^{1}H NMR Spectra in DMSO-d_6. DMSO-d_6 is a good solvent for observing ^{1}H NMR signals due to the OH protons of CDx.[21] Lightner et al. measured the CD spectra of BR in DMSO containing α- and β-CDxs, and found no bisignate CD Cotton effect.[15] In the present study, we measured the ^{1}H NMR spectra of β-CDx in DMSO-d_6 containing dianionic BR (BR^{2-}). The results are shown in Fig. 6. The protons of the OH groups at the 2-, 3-, and 6-positions of β-CDx (4×10^{-3} mol dm^{-3}) in DMSO-d_6 were observed at 5.73 (d), 5.68 (d), and 5.46 ppm (t), respectively. Upon the addition of equimolar amounts of BR^{2-}, the signals due to the OH protons at the 2- and 3-positions (OH-2 and OH-3) coalesce with each other and shift to a lower magnetic field (5.82 ppm). Meanwhile, the signal due to the OH proton at the 6-position of β-CDx (OH-6) is broadened, but does not shift upon the addition of BR^{2-}. These results clearly indicate the formation of hydrogen bonds between the OH groups at the 2- and 3-posi-

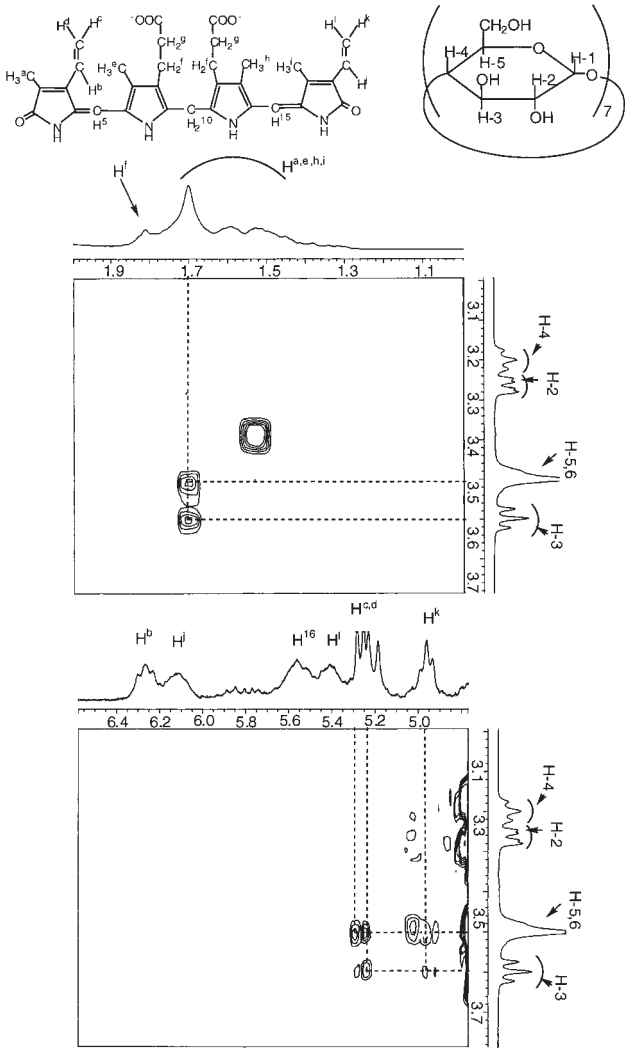

Fig. 5. ROESY spectrum of the BR^{2-}–β-CDx complex in D_2O at pD 10.8 and 5 °C. The concentrations of BR^{2-} and β-CDx are 0.020 and 0.016 mol dm^{-3}, respectively.

tions of β-CDx and the CO_2^- group(s) of BR^{2-}. Broadening of the signal due to OH-6 without a shift suggests the interaction of β-CDx and BR^{2-} other than hydrogen bonding between the OH group at the 6-position of β-CDx and the CO_2^- group of BR^{2-}. Similar to the OH-6 signal, other signals due to β-CDx are broadened without shifts upon complexation.

The ^{1}H NMR spectra of BR^{2-} (4×10^{-3} mol dm^{-3}) in DMSO-d_6 in the absence and the presence of β-CDx are shown in Fig. 7. Slight up-field shifts of the signals were observed with the NH protons of BR^{2-} ($\Delta\delta < 0.03$ ppm). No shifts were measured for other protons of BR^{2-} upon the addition of β-CDx. Slight up-field shifts of the NH protons may be ascribed to the partial dissociation of intramolecular hydrogen bonds between the CO_2^- and NH groups of BR^{2-} because of the formation of hydrogen bonds between the CO_2^- group(s) of BR^{2-} and the OH groups of β-CDx. All signals due to the OH protons of β-CDx in DMSO-d_6 were not affected by the addition of BR in the acidic form (CO_2H form). Such a result can be explained by the fact that the CO_2H group is not a good hydrogen-bond donor for β-CDx in DMSO-d_6.

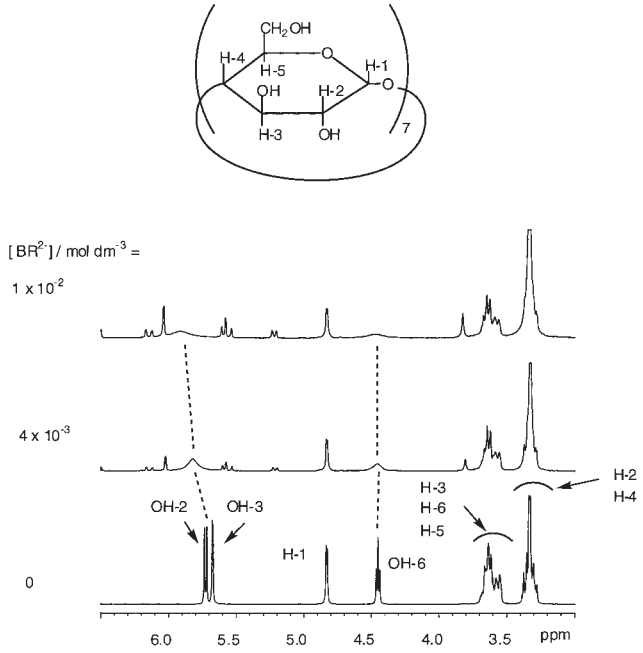

Fig. 6. ^{1}H NMR spectra of β-CDx (4×10^{-3} mol dm^{-3}) in DMSO-d_6 in the absence and the presence of BR^{2-} at 25 °C.

The simultaneous formation of two hydrogen bonds between a CO_2^- group of BR^{2-} and the OH groups at the 2- and 3-positions of β-CDx seems to be required to form a stable hydrogen-bonded complex, as in the case of the complexation of the *p*-methylbenzoate anion with β-CDx.[21] No correlation peaks were observed between BR^{2-} and β-CDx in the ROESY spectrum, suggesting that the BR^{2-} dianion hangs from the secondary OH groups of β-CDx through hydrogen bonding, and no penetration of the guest into the CDx cavity occurs in DMSO-d_6. Such a structure has been assumed for the *p*-methylbenzoate anion–β-CDx complex in DMSO-d_6.[23]

Although the ^{1}H NMR spectra indicate the formation of the hydrogen-bonded complex of BR^{2-} and β-CDx, no bisignate CD signals were measured in DMSO. This means the importance of the adsorption of the BR^{2-} dianion on the outside wall of the β-CDx cavity and/or the partial incorporation of BR^{2-} into the host cavity in the conformational enantiomerism.

Effects of *O*-Methylation of β-CDx. In a previous paper, we reported that TMe-β-CDx does not induce the conformational enantiomerism of BR^{2-}, while the effects of heptakis(6-deoxy)-β-CDx are essentially the same as those of β-CDx. A further study on the effects of the OH groups of CDx is needed for generalization.

At first, we used heptakis(2,6-di-*O*-methyl)-β-CDx (2,6-DMe-β-CDx) as a host. 2,6-DMe-β-CDx is commercially available. However, the purchased one contains about 50% impurities. The FAB MS spectrum indicates that the main impurities are $[M - H + CH_3]$ and $[M - 2H + 2CH_3]$, where M represents 2,6-DMe-β-CDx. We thus tried to purify 2,6-DMe-β-CDx by means of silica-gel column chromatography. Repeated chromatography afforded almost pure 2,6-DMe-β-CDx. As shown in Fig. 8a, no bisignate CD Cotton effect was observed for the BR^{2-}–2,6-DMe-β-CDx system. Hepta-

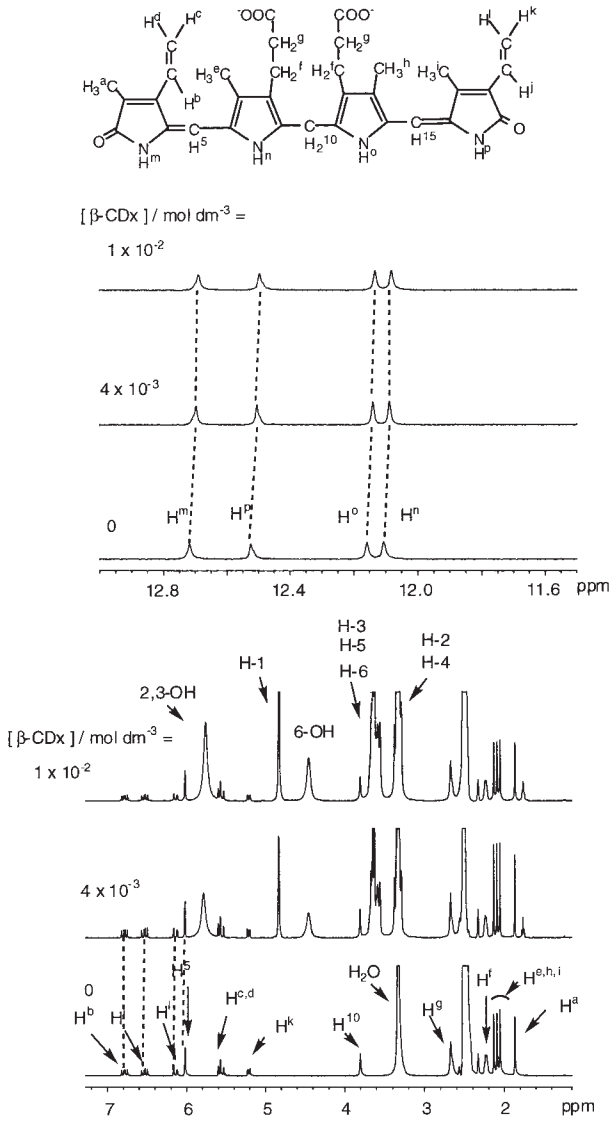

Fig. 7. ^{1}H NMR spectra of BR^{2-} (4×10^{-3} mol dm^{-3}) in DMSO-d_6 in the absence and the presence of β-CDx at 25 °C.

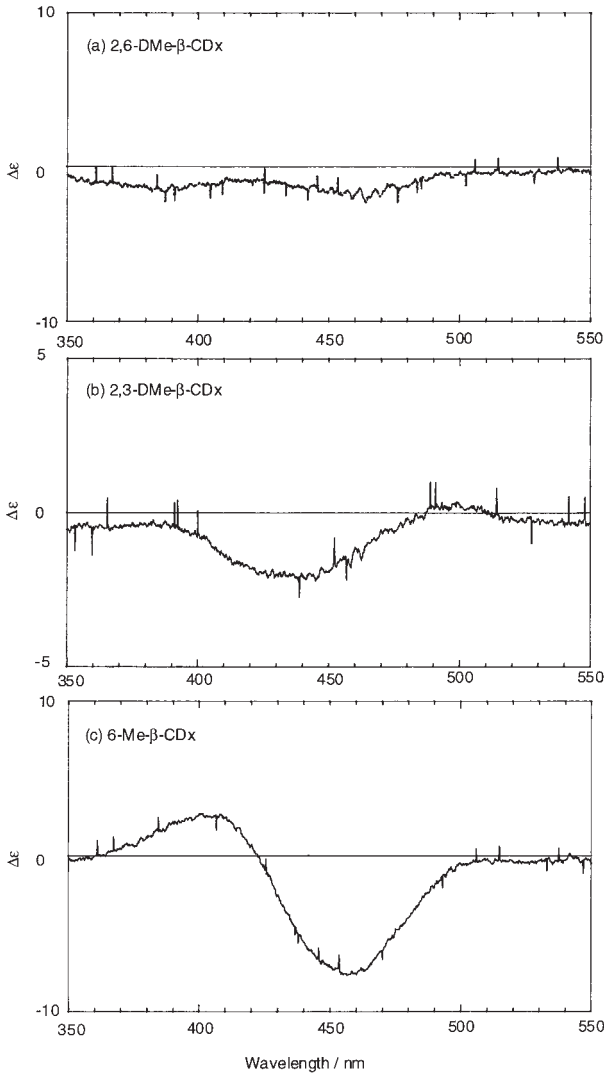

Fig. 8. CD spectra of BR^{2-} (2.5×10^{-5} mol dm^{-3}) in H_2O in the presence of alkylated CDxs (6×10^{-3} mol dm^{-3}) at pH 10.8 and 20 °C.

kis(2,3-di-*O*-methyl)-β-CDx (2,3-DMe-β-CDx) shows a simple, negative CD Cotton effect (Fig. 8b), suggesting the formation of a chiral BR^{2-} complex whose two dipyrrinone chromophores do not interact with each other through exciton coupling. On the other hand, a distinct bisignate CD spectrum was measured with the BR^{2-}–6-Me-β-CDx system (Fig. 8c). These results clearly indicate that two neighboring secondary OH groups at the 2- and 3-positions of β-CDx participate in the conformational enantiomerism of BR^{2-}.

Discussion

BR^{2-} is weakly bound to β-CDx, the K values being 23 dm^3 mol^{-1}.[16] In this work, the K value for the complexation of BR^{2-} with α-CDx at pH 10.8 and 25 °C was determined to be 15 ± 3 dm^3 mol^{-1} from CD spectral titration (see Experimental Section). The K value for the BR^{2-}–β-CDx complex increases in the presence of cyclooctanol ($K = 53$ dm^3 mol^{-1}).[16] As Table 1 shows, only alkanols that are tightly included into the CDx cavity cause an enhancement of the (−)-to-(+) bisignate CD intensity of BR^{2-}. It has been known that some alkanols act as space regulators to form stable guest-alkanol–CDx three-component complexes.[26,27] The CPK molecular model, however, suggests that there is no vacant space in the cavity of the cyclooctanol-loading β-CDx to place a BR^{2-} dianion deeply. All of the data listed in Table 1 definitely indicate that deep penetration of the BR^{2-} dianion is not essential for the enantioselective complexation of BR^{2-} with native CDx. The ^{1}H NMR data suggest that the BR^{2-} dianion is adsorbed on the outside wall of the β-CDx cavity. The CIS pattern of the β-CDx observed for the BR^{2-}–cyclooctanol–β-CDx three-component system (the data are not shown herein) is almost the same as that shown in Fig. 2, which is the CIS in the absence of cyclooctanol. Therefore, the structure of the BR^{2-}–CDx complex in the presence of cyclooctanol seems to be essentially the same as that in the absence of the alkanol. The ROESY spectrum, however, suggests the penetration of a hydrophobic part of BR^{2-} into the β-CDx cavity to some extent. Presumably, alkanols, such as cyclooctanol,

provide hydrophobic environments where a part of the BR^{2-} dianion is placed to form very shallow inclusion complexes. Shallow inclusion may cause propinquity of the CO_2^- group(s) of BR^{2-} to the secondary OH groups of β-CDx. The close location of the CO_2^- and OH groups and increased hydrophobicity of the host cavity may make it possible to promote a hydrogen-bonding interaction between BR^{2-} and CDx.

Before discussing hydrogen bonding between BR^{2-} and β-CDx in aqueous solution, let us consider hydrogen bonding in DMSO. The formation of two hydrogen bonds between a CO_2^- group of BR^{2-} and the OH groups at the 2- and 3-positions of β-CDx was undoubtedly confirmed from ^{1}H NMR measurements in DMSO-d_6 (Fig. 6). Meanwhile, no hydrogen bonds are formed between the acidic form of BR and β-CDx in DMSO-d_6. If two CO_2^- groups of BR^{2-} participate in complexation, enantioselective complexation can be expected, even in DMSO, because the asymmetrically twisted β-CDx cavity[28] can act as the third factor for chiral recognition through a three-point attachment model.[29,30] Presumably, no inclusion of the BR^{2-} dianion into the CDx cavity is the reason for complexation without stereoselectivity in DMSO. In any case, the results obtained for complexation in DMSO suggest the possibility of a hydrogen-bonded complex of BR^{2-} and β-CDx in aqueous solution.

It was found that 2,6- and 2,3-DMe-β-CDxs do not induce a conformational enantiomerism of BR^{2-}, though 6-Me-β-CDx shows the same effect as that of β-CDx (Fig. 8). These results reveal that two neighboring OH groups at the 2- and 3-positions of β-CDx are essential for promoting the conformational enantiomerism of BR^{2-}. An explanation of such a finding can be possible only by assuming two-point hydrogen bonds between the CO_2^- group of BR^{2-} and the secondary OH groups at the 2- and 3-positions of β-CDx. If hydrogen bonds do not participate in the conformational enantiomerism, 2,6-DMe-, 2,3-DMe-, and TMe-β-CDxs should act as chirality inductors. Recently, we found that CDxs, such as native CDxs and TMe-β-CDx, do not repel anionic guests, though they hate cationic ones.[31] Indeed, ^{1}H NMR measurements suggest interactions of the *O*-methylated CDxs with BR^{2-}. For example, all proton signals due to 2,6-DMe-β-CDx (4×10^{-3} mol dm^{-3}) shift to higher magnetic fields ($\Delta\delta < 0.04$ ppm) together with a slight broadening upon the addition of BR^{2-} (1×10^{-2} mol dm^{-3}). Similar shifts and broadening of the ^{1}H NMR signals were measured with 2,3-DMe-β-CDx ($\Delta\delta < 0.03$ ppm). These data suggest that the BR^{2-} dianion is adsorbed on the outside wall of the *O*-methylated CDx through van der Waals interactions, similar to the case of native β-CDx. However, no bisignate CD signal was observed in the cases of 2,6- and 2,3-DMe-β-CDxs. This means that a special interaction between the CO_2^- group of BR^{2-} and the secondary OH groups of native CDx is needed for conformational enantiomerism. The only plausible interaction is hydrogen bonding. Coexisting alkanol seems to enhance the hydrophobicity near the rim of the CDx cavity. A hydrophobic environment is preferable for forming a hydrogen bond. An image of a chiral BR^{2-} complex of β-CDx in the presence of cyclooctanol is shown in Fig. 9. The BR^{2-} dianion is anchored by hydrogen bonding between the CO_2^- group(s) of BR^{2-} and the secondary OH groups of β-CDx, and is adsorbed on the β-CDx cavity through van der Waals interactions. The asymmetrically twisted β-CDx cavity causes preferential binding of (*M*)-helix BR^{2-} to the β-CDx–cyclooctanol complex. The ROESY spectrum suggests that another type of the complex is also formed. The hydrophobic part of such a second complex is placed at the primary OH group side of β-CDx. Therefore, it is impossible to form hydrogen bonds between the secondary OH groups of β-CDx and the CO_2^- group(s) of BR^{2-} bound to the primary OH group side. As a consequence, BR^{2-} taking geometry other than that shown in Fig. 9 might be achiral. The conformational enantiomerism of BR^{2-} induced by nucleotides,[12] octyl β-D-maltoside micelles,[13] non-cyclic oligosaccharide,[14] and steroid[20] might be interpreted in terms of the same mechanism presented in this study.

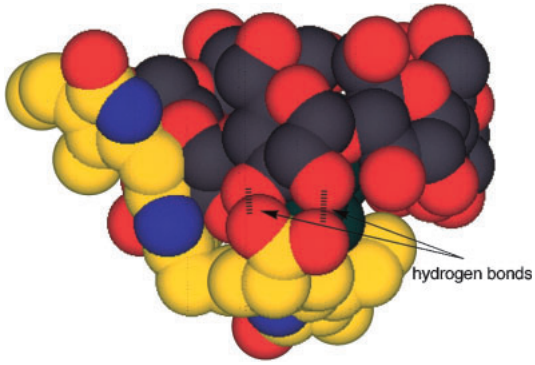

Fig. 9. Image of the BR^{2-}–cyclooctanol–β-CDx complex. The carbon atoms of β-CDx, BR^{2-}, and cyclooctanol are represented by gray, yellow, and green colors, respectively.

Experimental

Native α- (Wako), β-, and γ-CDxs (Nacalai) were purchased. β-CDx was used after washing with THF using a Soxhlet extractor. 2,3- and 2,6-DMe-β-CDxs were prepared according to the procedures described in the literature.[32] Purification of 2,3-DMe-β-CDx was performed by silica-gel column chromatography with chloroform–methanol (8:1 to 4:1). The FAB MS spectrum of the purified 2,3-DMe-β-CDx indicates that no other impurities exist. Mp 165–169 °C; FAB MS (*m*-NBA matrix) *m*/*z* 1354 ($[M + Na]^+$); Anal. Calcd for $C_{56}H_{98}O_{35}\cdot 2H_2O$: C, 49.19; H, 7.52; O, 43.29%; found: C, 49.14; H, 7.33; O, 42.98%. The purification of 2,6-DMe-β-CDx was performed by repeated silica-gel column chromatography (5 times) with chloroform–methanol (20:1). The FAB MS spectrum of 2,6-DMe-β-CDx before purification indicates that the main impurity is the CDx having one extra OCH_3 group ($[M - H + CH_3]^+$), which could be removed by repeated silica-gel column chromatography. After several treatments for purification, small amounts of impurities were still remaining. Mp > 285 °C (decomp); FAB MS (*m*-NBA matrix) *m*/*z* 1354 ($[M + Na]^+$); Anal. Calcd for $C_{56}H_{98}O_{35}$: C, 50.52; H, 7.42%; found: C, 49.84; H, 7.31%. Purification of the purchased BR (Sigma) was achieved by a reported method.[15] Namely, BR in the acidic form was dissolved in chloroform and washed with 5% aqueous $NaHCO_3$ (5-times). The chloroform solution was dried on Na_2SO_4, and the solvent was removed under reduced pressure. The residue was washed with a small amount of methanol. The disodium salt of

BR^{2-} was obtained by neutralization of BR in the acidic form in methanol with 0.1 mol dm^{-3} aqueous NaOH. After neutralization, methanol was removed under reduced pressure and the residue was washed with chloroform. All treatments were carried out under shading conditions. Other materials were purchased and used without purification.

The CD spectra were measured using a JACSO J-500 spectropolarimeter with a data processor at 25 °C. The pH value was adjusted by aqueous NaOH to be 10.8. No significant change in the pH was confirmed after each measurement. The concentrations of BR^{2-} and CDx were 2.5×10^{-5} and 1×10^{-2} mol dm^{-3}, respectively. The effects of coexisting alkanols were examined in the presence of 1×10^{-2} mol dm^{-3} alkanols. The ^{1}H NMR spectra (400 MHz) were taken on a JEOL GX 400 spectrometer using 3-trimethylsilyl[2,2,3,3-d_4]propionate (TSP, Aldrich) as an external standard. FAB MS spectra were recorded on a JEOL MStation JMS-700 spectrometer (positive mode) using *m*-nitrobenzyl alcohol (*m*-NBA) as a matrix.

The K value for the complexation of BR^{2-} with α-CDx in aqueous solution at pH 10.8 was determined from a titration curve obtained by plotting the CD intensities of BR^{2-} vs [α-CDx]. The titration curve was analyzed by a nonlinear least-squares method to evaluate the K value (15 ± 3 dm^3 mol^{-1}).

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